Polyurethane scaffolds as meniscus reconstruction materials

Heijkants, Ralf Guillaume Jean Catharina

8

Degradation of 1,4-butanediisocyanate and poly(ε-caprolactone) based polyurethanes. Part I: influence of soft segment length

R.G.J.C. Heijkants, R.V. van Calck, J.H. de Groot, A.J. Pennings and A.J. Schouten

Abstract

Polyurethanes based on poly(ε-caprolactone) (PCL) and 1,4-butanediisocyanate with different soft segment lengths and constant uniform hard segment length were hydrolytically degraded in phosphate bufferd saline at 37°C. The degradation proceeded as expected for bulkdegradation although an additional degradation mechanism (see Part II) showed up. The rate of degradation was of the same order as for the homopolymer PCL and PU1600>PU2300>PU2800 and the hard segment melting point of PU1000 and PU1600 remained constant. PU2300 and 2800, however, showed an increase in hard segment melting point of about 10°C. Except for PU1000, all samples showed some increase in soft segment crystallinity upon degradation.

The mechanical properties of the films decreased monotonically until the additional degradation mechanism prevailed. At that point the mechanical properties decreased drastically.

Introduction

Since a long time polyurethane elastomers have been used in various medical applications varying from blood bags to implantable devices like vascular and meniscus prosthesis [1-4]. Polyurethanes are very suitable for these applications due to their excellent physical and mechanical properties in combination with a high biocompatibility. These properties originate from the phase-separated structure that this type of polymers possesses. By changing, among others, the ratio and/or the type of blocks that are used a very broad spectrum of properties can be obtained.

All of the current commercial applications that use polyurethanes are based on polymers that are meant to be biostable [5]. These polymers are generally based on an aromatic hard segment and a soft segment of a polyether or polysiloxane polyol. In case of applications which require a (bio)degradable polyurethane the soft segment is mostly based on a polyester which makes the material sensitive for hydrolytic scission although one should not forget biodegradation mechanisms like enzymatic degradation can also play a role [1].

The major additional point of attention is the biocompatibility of the polymer fragments formed upon degradation. The soft segment part of a polyurethane generally does not yield toxic compounds upon degradation. In contrast to this hard segments are generally based on aromatic diisocyanates, which do release toxic diamines on degradation. Even most aliphatic diisocyanates are known to release toxic compounds on degradation [6-9]. Exceptions to this are polyurethanes with a hard segment based on 1,4-butanediisocyanate (BDI). Upon degradation these polymers are expected to release 1,4-butanediamine which is also known as putrescine, a natural polyamine which is a precursor for spermidine and known to enhance cell growth [10]. Moreover, in contrast to 1,6-hexanediisocyanate, BDI based polymers were found not to have an ecotoxicological impact, which also suggest good biocompatibility with respect to the other polyurethanes [11]. The soft segment is based on poly(ε-caprolactone) (PCL) which is well known for its biocompatibility, slow degradability and is already used in long-term release systems [12]. Besides products that originate from the polymer chain the generally used catalysts for the synthesis of polyurethanes which besides their influence on the degradation are also not considered to have a positive effect on the body [13,14]. For this reason the materials here are made in absence of any catalyst.

To examine the degradation properties of the polyesterurethanes of Chapter 2 and the foams thereof (Chapter 6) four polyurethanes based on different poly(ε-caprolactone) soft segment lengths and constant hard segment size were subjected to hydrolytic degradation at 37°C in phosphate buffered saline solution. Beside intrinsic viscosity and molecular weight, the thermal properties and mechanical properties were measured as a function of the degradation time.

Experimental

Materials and methods

Dioxane (Merck) was distilled from sodium. Dimethylsulfoxide (DMSO, Acros) was distilled from CaH_2 under reduced pressure. Petri dishes were siliconized with a silicone solution from SERVA (Heidelberg, Germany) before use followed by drying at 130ºC. The phosphate buffered saline solution (PBS) was obtained from the pharmacy of the university hospital of Groningen, Sodium azide (Merck) was used as received.

A Perkin-Elmer DSC-7 was used for thermal analysis operated under a nitrogen atmosphere. Samples of 3-8mg were heated from –100°C to 150°C at a heating rate of 10^{o}C/min. Tensile tests were performed using rectangular (25x2.2x0.1mm) shaped specimens cut from the solvent cast film. Tests were performed at 21°C with a 100N load cell and an extension rate of 10mm/min and a grip-to-grip distance of 20mm using an Instron 4301 tensile tester. The values obtained are an average of at least three samples. Compression measurements were performed at room temperature and at a compression rate of 2mm/min with samples of about 0.5x0.5x0.5cm. The compression moduli were determined at 20% compression of the foams.

Gel Permeation Chromatography (GPC) measurements were performed in dimethylformamide with 0.01M LiBr on a Waters 600 Powerline system, equipped with 2 mixed-C Plgel 5μ columns (Polymer Laboratories) kept at 70°C. The data-analysis was done using conventional calibration with polystyrene standards.

The intrinsic viscosity of the polymers was determined at 25°C using a type Oa Ubbelohde viscometer with chloroform as solvent.

A Jeol 6320 F Field Emission Scanning Electron Microscope (FESEM) was used for studying the pore structure of the porous materials. It was operated at a working distance of 11mm, an acceleration voltage of 5kV and a beam current of $1 \cdot 10^{-10}$ Amperes. The specimens were made conductive with a 3nm layer of gold using a Cressington Rotating Magnetron Sputter Coater operated at a working distance of 150mm and a current of 20mAmperes.

Polyurethanes

The polyurethanes used here are based on a soft segment of poly(ε-caprolactone) initiated on 1,4-butanediol (PCL) synthesized without catalyst (1000, 1600, 2300 and 2800g/mol). The hard segment is based on 1,4-butanediisocyanate and 1,4-butanediol and has a uniform length. The synthesis is described in Chapter 2, but performed with a polymerization time of 16 hours. PUx indicates the polyurethanes with x as the molecular weight of the PCL segment.

Foams

Foams were made from all four polymers. The method was the same for all polymers and described in Chapter 6. The obtained scaffolds had a porosity of about 83%. Figure 8-1 shows the structure of the foam based on PU1600.

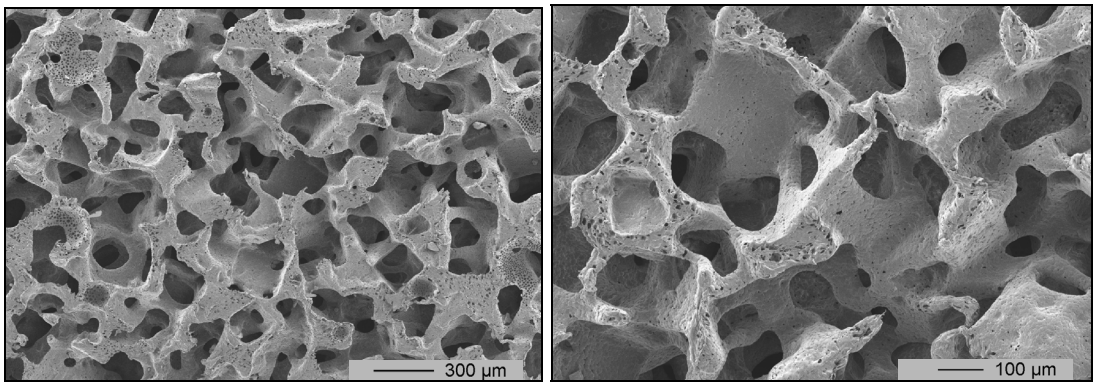

Figure 8-1. PU1600 foam structure with a porosity of 83% and a compression modulus of 350kPa.

Polymer properties and film preparation

Polymer films were obtained by dissolving foams of the different polyurethanes in dioxane in a concentration of 2wt%. This solution was cast in a petri dish that has been pretreated with a silicone solution of SERVA to prevent the film from sticking to the glass. The solvent was evaporated at room temperature (PU1000 was cast at 60°C) after which the film was dried further in a vacuum stove at 50°C for 24 hours to remove the last traces of dioxane. The films had a thickness of about 0.1mm. Before use the films were cut in quarters. The hard segment content, GPC data, intrinsic viscosity, glass transition and melting points of the initial properties are summarized in Table 8-1.

Table 8-1. Initial properties of the polyurethane films.

Polymer code	Hard segment content (%)	M_n (kg/mol)	PDI (-)	Intrinsic viscosity (dl/g)	T_g (°C)	T_m PCL (°C)	T_m hard segment (°C)
PU1000	27	80	1.8	0.74	-49	-	114
PU1600	19	115	2.2	1.35	-56	-	94
PU2300	14	107	2.3	1.21	-59	31	71
PU2800	12	132	2.6	2.03	-60	32	68

-: not present

During degradation the samples were kept in the dark at 37°C in 400ml phosphate buffered solution containing 0.02wt% sodiumazide (to prevent biological contamination) per four samples. At each analysis point a sample was withdrawn, blotted, weighed, dried for 24 hours at 37°C in a vacuum stove and weighed again.

Together with the initial weight before degradation the degree of swelling and mass loss were determined according the following relationships:

$$Weightloss = \frac{(M_o - M_d)}{M_o} \times 100\% \quad (1)$$

with: M_0 = mass before degradation and M_d = mass after drying.

$$Degree\,of\ swelling = \frac{(M_w - M_d)}{M_o} \times 100\% \quad (2)$$

with: M_0 = mass before degradation and M_d = mass after drying and M_w = mass of wet film.

Results and discussion

Visually none of the polymers showed any change in sample dimensions while also no change in pH of the PBS solution was noted. During the start of the experiment all films were clear or were slightly opaque depending on the polymer. PU1600 and PU2300 were clear, while PU1000 and PU2800 were slightly opaque due to the amount of crystalline hard and/or soft segment present. After some time some films showed opaque spots or turned completely opaque although this was never seen with PU2300 (Figure 8-2). These spots showed significantly different properties and thus for as far as possible measurements were performed both on clear and opaque spots. The degree of swelling was determined for the clear films, opaque parts of films were too fragile to determine the degree of swelling. The clear films showed hardly to any change in swelling. For all polymers the value remained beneath 2%. The origin of the opaque spots and the accompanying properties of the films will be treated in Part II.

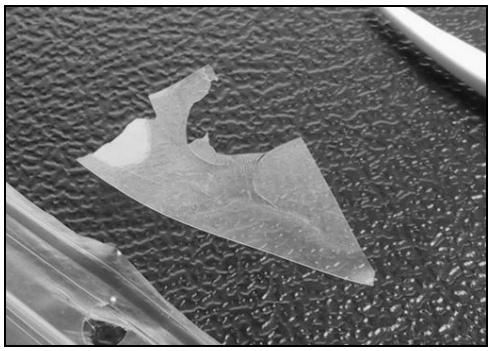

Figure 8-2. Example of inhomogeneous degradation of polymer film

Figure 8-3 shows the change in intrinsic viscosity with change in time. These graphs visualize one of the main differences between clear and opaque spots (see Part II).

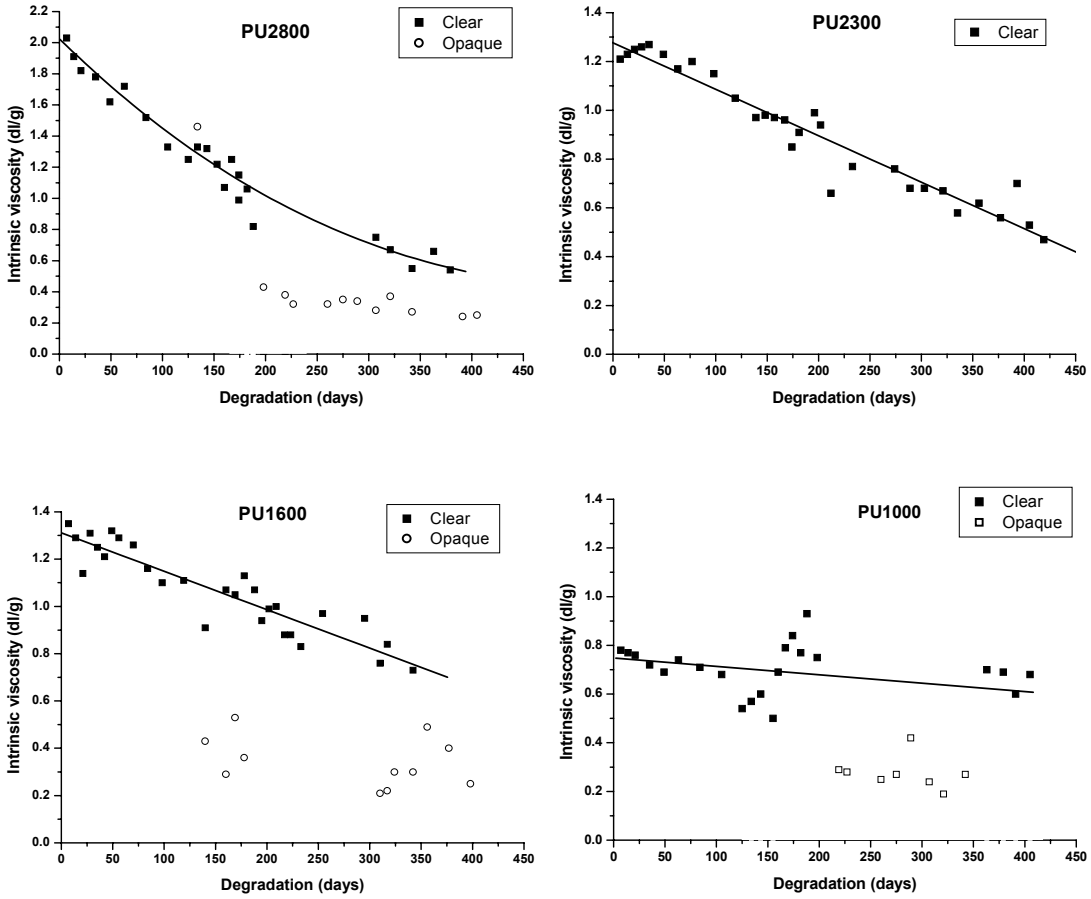

Figure 8-3. Change of intrinsic viscosity with degradation time for PU2800, 2300, 1600 and 1000.

With GPC the same trend was found. In literature it is (generally) accepted that chain scission in polyesterurethanes generally occurs at the ester groups since the urethane groups are found to be far less sensitive for hydrolytic scission [15-17]. If only chain scission by ester hydrolysis is considered (and urethane scission is neglected) here too then for bulk degradation there are two generally accepted mechanisms: autocatalysis and rate law degradation. If the degradation follows the rate law the following equation represents the change in molecular weight with time [18]:

$$\frac{1}{M_n} = \frac{1}{M_n^o} + k't \qquad (3)$$

in which M_n is the average number molecular weight, M_n^o the initial average molecular weight, k' the rate constant and t the time of degradation. Hydrolysis of ester groups can also be catalyzed by carboxylic end groups. Based on this a first-order kinetic model from Pitt [19] related the rate of chain scission autocatalyzed by carboxylic end groups formed during the degradation to the decrease of molecular weight in time via the following relation: $\ln(M_n) = \ln(M_n^o) - kt$ in which k the autocatalytic rate constant. Both these mechanisms assume that there is no loss of weight as was found for the clear films.

With this assumption we calculated the degradation rate constants on the basis of the clear parts of the films for both catalyzed and uncatalyzed models (Table 8-2). Except for PU1000 a very good fit is found although without a significant difference between both methods. The values found for the rate constants are in the same order as is found in literature for PCL [19,20]. A slight deviation is found, which might be related to the difference in PCL/soft segment crystallinity compared to high molecular weight PCL [17]. It was found that the rate constant decreases in the order of PU2800> PU2300> PU1600.

If PU2800 is compared to the homopolymer PCL, it degrades slightly faster than the homopolymer. This can be attributed to the difference in PCL crystallinity, which is significantly higher for PCL (if it is assumed that the urethane groups are not sensitive to degradation). Water cannot penetrate crystals which will thus make less ester groups available for hydrolysis [21,22]. Although it should be mentioned that this conclusion depends on the literature values for PCL that are used for comparison. The one mentioned in Table 8-2 is $3.1 \cdot 10^{-3}$/day while Pitt [23] found a rate constant of $1.8 \cdot 10^{-3}$/day which is significantly lower. In this case not only PU2800 degrades faster, but also PU1600 and PU2300 degrade faster compared to PCL.

Table 8-2. Calculated degradation rate constants for autocatalytic and rate law degradation.

Polymer	Rate constant ($\cdot 10^3$ day^{-1})	
	autocatalytic (k)	rate law (k')
PU1000	0.188±0.262	0.267±0.395
PU1600	1.57±0.136	1.54±0.209
PU2300	2.22±0.118	2.73±0.183
PU2800	3.42±0.158	3.44±0.209
PCL	3.1 [19,20]	

In contrast to what has been found here it has also been mentioned in literature that polyesterurethaneurea's based on poly(ε-caprolactone) and BDI and 1,4-butanediamine (instead of the 1,4-butanediol as has been used here) show almost a halving of the molecular weight after 56 days of degradation in combination with a few percent of weight loss [24]. This is much faster compared to our findings. This suggests that the hard segment can have a major influence the rate of degradation. This is in sharp contrast to the general idea in literature that urethane groups are far less sensitive for hydrolysis compared to ester groups. In principle it might also be the case here that compared to the soft segment, the non-crystalline urethane groups are more sensitive to hydrolysis. In that case one would expect the same order of rate constants since the fraction of crystalline hard segment decreases with decreasing built in hard segment (see Chapter 2) which will thus make the PU2800 the most sensitive for hydrolysis and PU1000 the least sensitive. Generally also a higher degradation speed is expected for polymers that take up more water. In case of these polymers it was found that there is an increase in the degree of swelling with

increasing hard segment content (Table 2-8). This would suggest that in case of ester hydrolysis the higher hard segment PU's would degrade faster since the concentration of ester groups in the amorphous phase remains more or less constant while the amount of water increases, but the opposite is found. This suggests that the water concentration is not the limiting factor here. The only factor that increases with decreasing hard segment content is the percentage of amorphous hard segment, which might thus be the basis for the rate of degradation.

Thermal properties

The thermal properties of the polymers were followed using differential scanning calorimetry. For the different PUx's it was already reported that the hard segment melting temperature depends on the hard segment content, a higher hard segment content leads to an increase in melting point (Table 8-1) [25] .
Figure 8-4 shows the melting points of PU1600 as a function of degradation time. As can be seen the melting point of the hard segment remains constant during the complete period of degradation and no difference is found between opaque and clear parts of the films (see Part II). PU1000 showed the same pattern, a constant hard segment melting point around 115°C, independent of clear and opaque parts of the film (not shown).

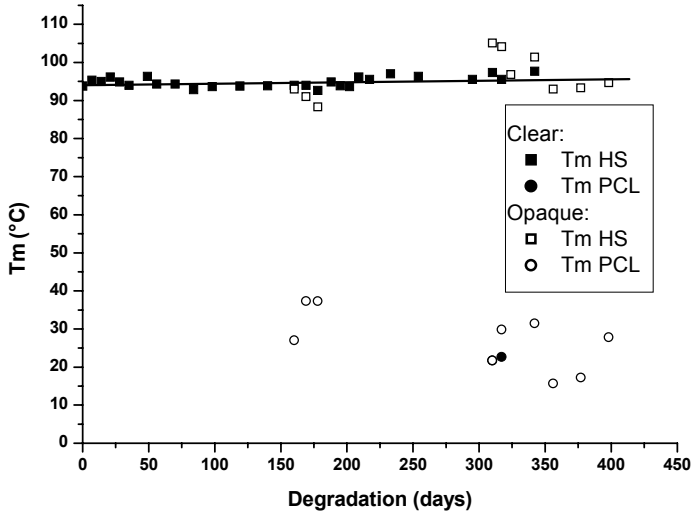

Figure 8-4. Melting points of hard segment and PCL of PU1600 determined from the first DSC heating scan.

For both PU2300 and 2800 a slight increase in hard segment melting point is found, although no difference is found between opaque and clear parts of the films (Figure 8-5 and Figure 8-6). During the complete degradation experiment the melting point of the hard segment increased about 10°C for both polymers. Since the increase in temperature already starts at the beginning of the experiment and is independent of opaque and clear parts of the film, annealing effects are the most likely basis of the increase.

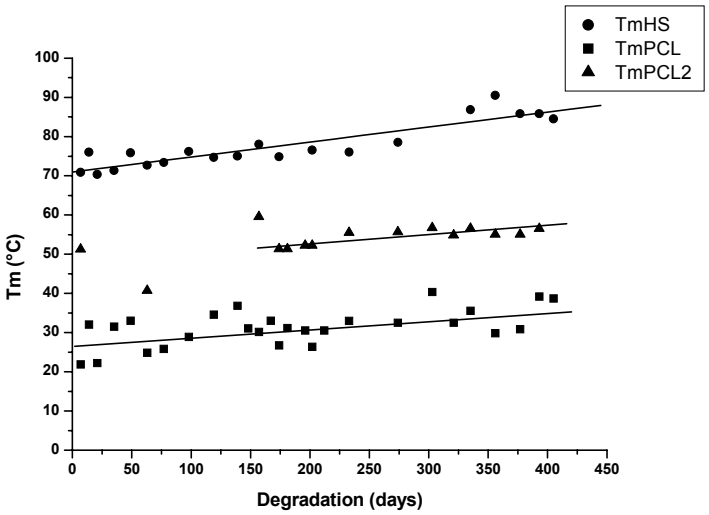

Figure 8-5. Melting points of hard segment (HS) and PCL soft segment of PU2300 determined from the first DSC heating scan.

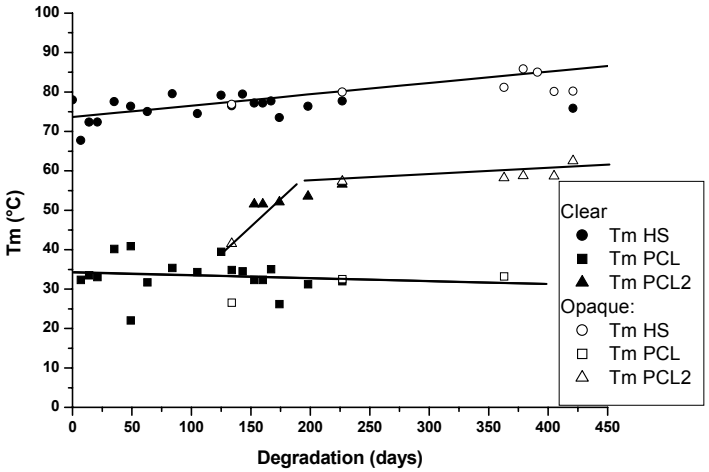

Figure 8-6. Melting points of hard segment (HS) and PCL soft segment of PU2800 determined from the first DSC heating scan.

However, the soft segment PCL can be(come) crystalline also. In case of PU1000 and 1600 initially there is probably too much hindrance of the (crystalline) hard segment to enable the soft segment to crystallize and no crystalline PCL was present at the start of the degradation. During the experiment no crystalline PCL was found for PU1000. Some opaque PU1600 samples showed a minor amount of crystalline PCL with a melting point above 37°C (Figure 8-7). In this case sufficient ester bonds are hydrolyzed to increase mobility to such an extent that PCL is able to crystallize. The melting point of this crystalline PCL is around 54°C. The melting enthalpy of these peaks found after 300 days of degradation are around 0.5J/g. If 136J/g is used as the melting enthalpy for 100% crystalline PCL and the values are corrected for the initial amounts for PCL present in the polymer the values shown in Figure 8-7 are obtained [26]. For PU1600 only 0.45% of crystalline PCL is present after 53 weeks of

degradation. The total ΔH of the crystalline PCL that was found using DSC is shown in Figure 8-7.

Besides this melting point, crystalline PCL already is found at earlier stages in the degradation experiment, but this PCL melts at temperatures below 37°C and will thus not be present as crystalline material in the body and will not influence the degradation and will not be accounted for. PU1000 never showed any crystalline PCL with a melting point above 37°C during the complete degradation experiment.

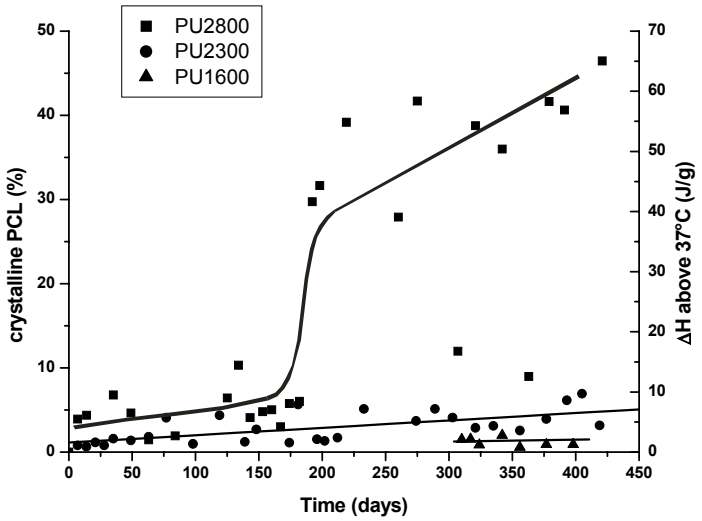

Figure 8-7. The amount of crystalline PCL present at 37°C plotted as percentage crystalline PCL and heat of fusion of crystalline PCL as a function of time for PU2800, 2300 and 1600.

In contrast to PU1000 and 1600, PU2300 and 2800 do initially show an additional melting point around body temperature due to crystalline PCL. Upon degradation PU2300 and 2800 both showed a slow increase in PCL melting temperature while after about 150-175 days of degradation a second PCL melting peak slightly above 50°C is found.

The PCL crystallinity of PU2300 increased from initially about 1% to almost 5% after 400 days of degradation (Figure 8-7). In contrast to this PU2800 shows a major increase in percentage of crystalline PCL. Before 175 days of degradation only about 5% of PCL is crystalline, but after this moment a sudden increase is found to almost 30% which slowly increases to 45% of crystalline PCL after 400 days of degradation.

This major increase in crystallinity might be disadvantageous. It is known that the degradation and dissolution of crystalline PCL is a prolonged process, while it has also been seen that crystalline debris formed during degradation may even cause undesired inflammatory responses [12,19,27].

The glass transition temperatures found for the different polyurethanes are all very low and initially between –49°C for PU1000 and –60°C for PU2800. The degradation hardly influenced the T_g's of PU1000, 1600 and 2300, which suggests that there is no change in crosslink density and phase mixing. On the contrary PU2800 showed an

increase from –60°C to about –50°C after 360 days of degradation due to an increase in PCL crystallinity.

Foam degradation

Foams of PU1600 with a porosity of 83% were also subjected to in vitro degradation. As can be seen from the SEM pictures of Figure 8-8, there is no apparent influence of the degradation on the structure of the scaffold. During the complete degradation experiment the scaffolds did not show any change in structure. Even after 41 weeks of degradation no major change in structure was found even though the foam was very delicate and difficult to cut without destroying the foam structure. Scaffolds of later periods in the degradation experiment were not suitable for SEM pictures, these foams crumbled on touching.

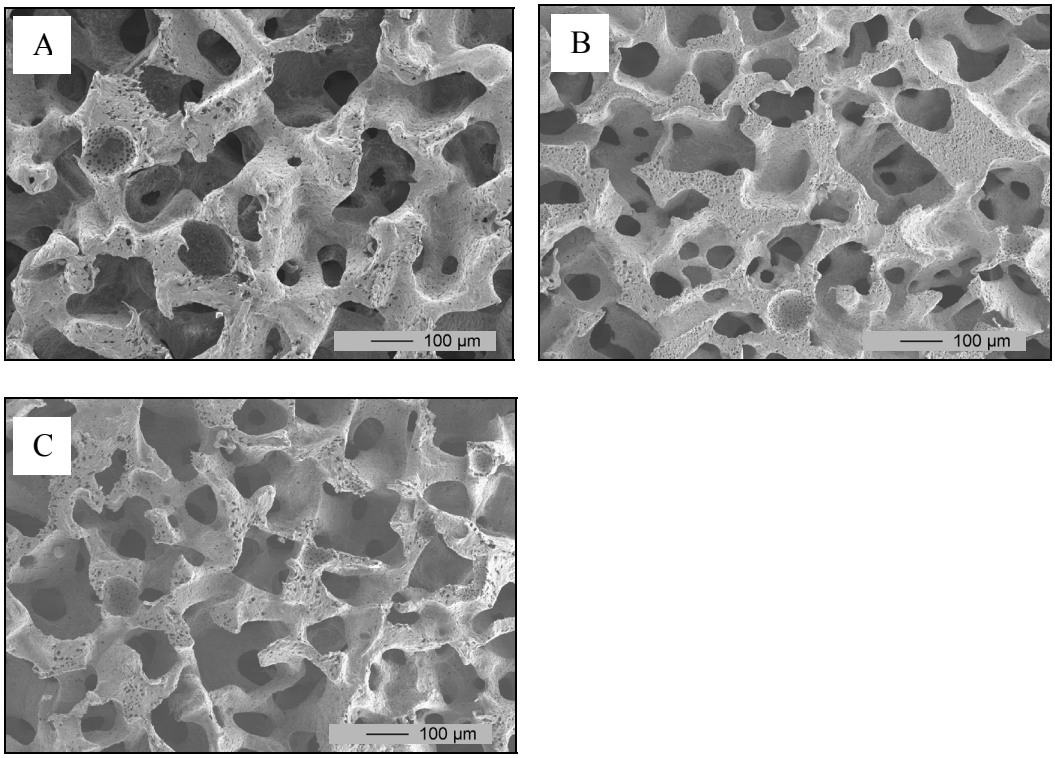

Figure 8-8. SEM pictures of degraded polyurethane scaffolds after different times of degradation a: 0 weeks b: 22 weeks c: 41 weeks

Compression modulus

Upon degradation there seems to be a slight increase of the compression modulus although this might be within the experimental error margin. If the scaffolds after 295 days of degradation were subjected to a small sideways stress, they completely crumbled. Unexpectedly, it was still possible to determine a compression modulus of these materials. These values did not deviate from the foams, which still possessed mechanical properties.

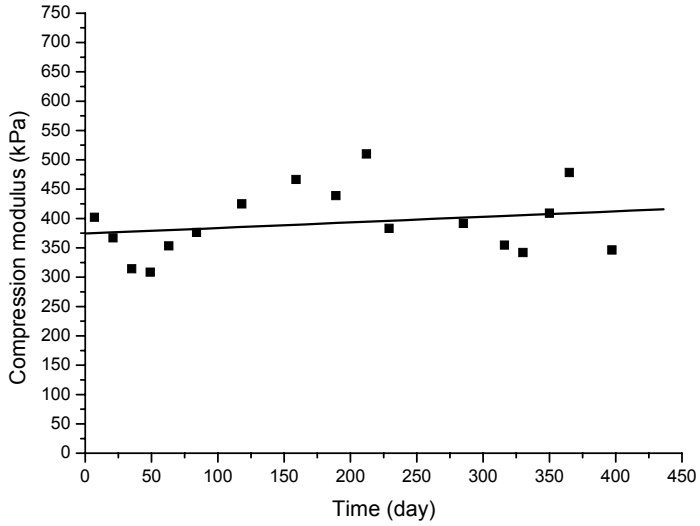

Figure 8-9. Compression modulus of PU1600 scaffold as a function of degradation time.

Molecular weight

GPC measurements were performed to be able to relate the degradation of the polymer films to the foams. The different physical background in combination with its porosity and different volume to surface ratio is known to influence the speed and type of degradation [28-30].

Figure 8-10 shows the molecular weights measured for PU1600 films and scaffolds as a function of the time of degradation. The measurement after 310 days of degradation was performed for a clear and opaque part of the film: the high molecular weight is from the clear part of the film while the low molecular weight originates from the opaque part. As can be seen in the graph the scaffold shows a comparable loss of molecular weight suggesting that the films are a good representation for the change in properties of the foams (see Part II).

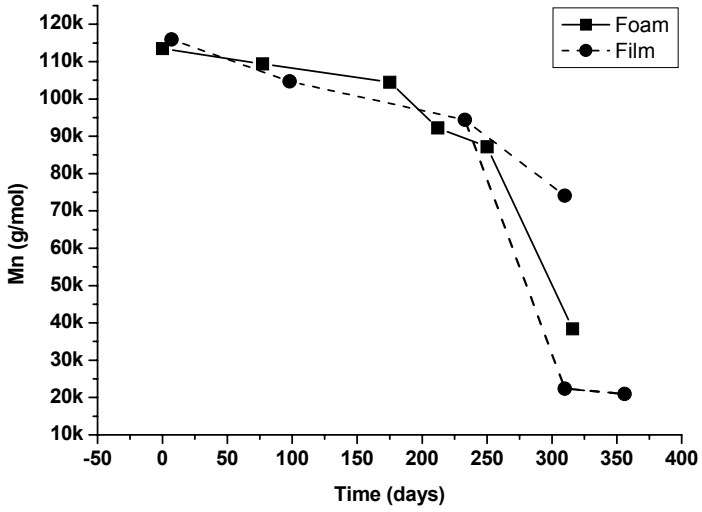

Figure 8-10. Number average molecular weight of the PU1600 film and foam versus time of degradation. The double point for the film at 310 days of degradation originates from a clear (upper point) and opaque (lower point) part of the film.

Mechanical properties

Stress and strain at break are mainly dependent on the molecular weight. Figure 8-11 shows the strain at break the intrinsic viscosity. A clear decrease in strain at break is noted for all polymers with decreasing intrinsic viscosity. Stress at break shows the same trend (not shown). From these graphs we estimated the minimum intrinsic viscosity needed to have mechanical properties (Table 8-4). This is roughly the same intrinsic viscosity as where the resistance to tear became zero for PU1000 [25].

The mechanical testing was performed on samples that were either completely clear or completely opaque. Table 8-3 shows the initial tensile properties as a function of the different polymers.

Table 8-3. Initial mechanical properties of the PUx polymers.

	Young's modulus [MPa]	Stress at break [MPa]	Strain at break [%]
PU1000	155	29.3	790
PU1600	63	30.2	1127
PU2300	42	31.7	1132
PU2800	102	33.2	1070

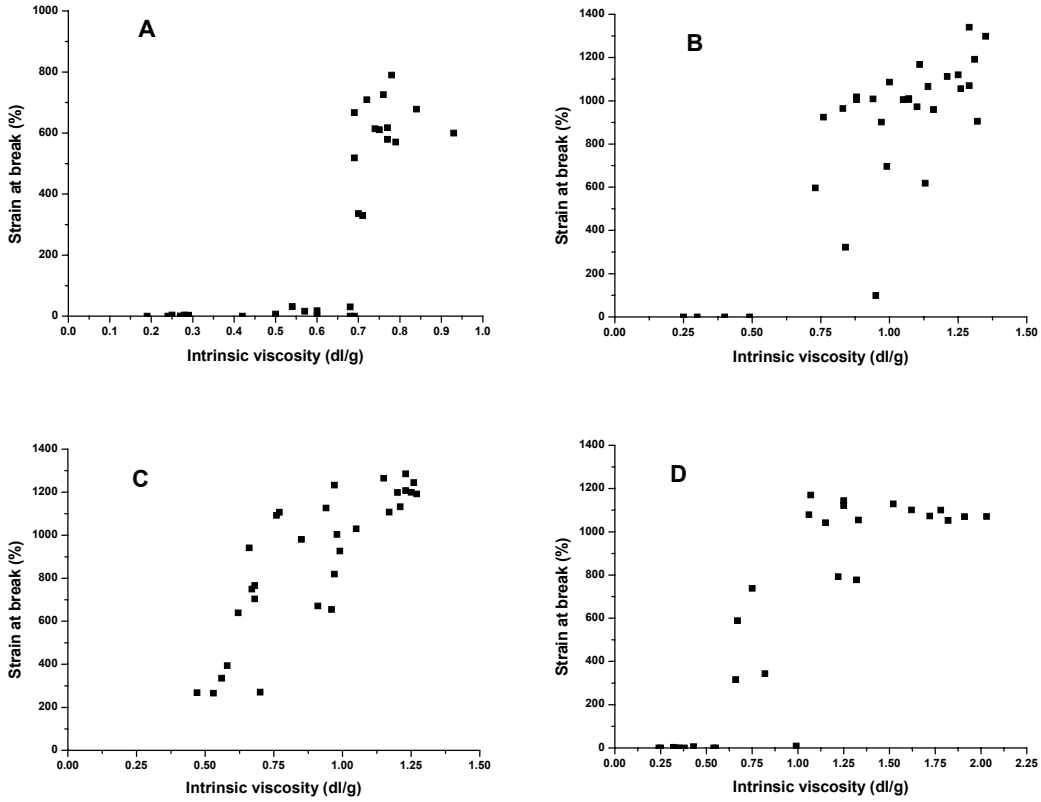

Figure 8-11. Strain at break versus intrinsic viscosity A:PU1000, B:PU1600, C:PU2300, D:PU2800.

Table 8-4. Estimated minimum intrinsic viscosity values needed for a minimum of mechanical properties.

	Minimum intrinsic viscosity	
	according to stress at break	according to strain at break
PU1000	0.4-0.5	0.6-0.7
PU1600	0.5-0.75	0.5-0.75
PU2300	0.4-0.5	0.4-0.5
PU2800	0.5-1.0	0.6

The Young's modulus shows a different trend. As shown in Chapter 2 it is mainly dependent on the amount of crystalline material in the sample. In Figure 8-12 it is visible that a small decrease with time is noted for PU1000 while for PU1600 and PU2300 an increase in Young's modulus is found with time, although PU2300 shows a decrease in modulus after 300 days of degradation related to the increase in crystallinity with time. The Young's modulus of PU2800 was not suitable for analysis since there is a variable amount of PCL that crystallizes during cooling from 37°C to room temperature (see Figure 8-3) which lead to major variations in the obtained results (40-160MPa).

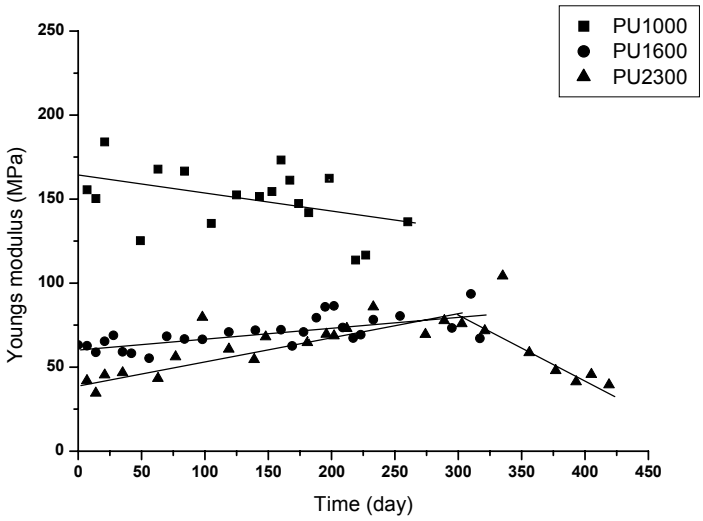

Figure 8-12. Young's modulus versus time of degradation.

Conclusions

Four different PUx's (PU1000, 1600, 2300 and 2800) were fist processed into foams and subsequently dissolved in dioxane [31]. From these polymer solutions films were cast and these films were used to study the in vitro degradation in PBS buffer at 37°C. It was found that the water uptake is relatively low for all polymers and remains below 2% during the complete degradation experiment. Upon degradation it was noted visually that the films of PU1000, 1600 and 2800 did not degrade homogeneously. After a certain period some films showed opaque spots or became completely opaque (see Part II). The parts of the films that remained visually unchanged showed a decrease of molecular weight comparable to the homopolymer PCL.

Thermal analysis of the materials showed no change for PU1000. For PU1600 an appearance of a minor amount of crystalline PCL during the end of the experiment was found, while PU2300 and especially PU2800 showed a major increase in crystalline PCL.

It was found that the polymers degrade with a comparable rate as the homopolymer PCL, while the films are a good representation for the foams. The polymers lost their mechanical properties around the same intrinsic viscosity, about 0.5 to 0.7dl/g. This took from several months for PU1000 to more than a year for PU2300. The compression modulus of the foams was retained during almost the complete period. If foams, comparable in degradation time with opaque films, were subjected to a minor sideways force, they crumbled.

Overall, these polymers can be very suitable for applications for which long-term mechanical properties are combined with degradability like a meniscus scaffold.

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